culiar cup-shaped membrane, is very characteristic of this bacillus, certainly as reassuring to the observer as the stellate growth of its colonies on gelatine. For if another microbe be present, the liquid remains clouded and the membrane is modified. There are many other points of interest in connection with the membrane and the deposit formed later, which I forbear to mention here, as their value for diagnostic purposes is limited by their tardy appearance.

If we now turn our attention to solid media, we shall find that diagnosis often fails when the bacteria do not liquefy the substratum. The colonies then are apt to develop very much alike, and any modification may as well be credited to changes in the consistency of the gelatine, due to variations in temperature, to drying, etc. These same changes will modify the appearances of colonies of the same microbe. Tube cultures in gelatine of radically different bacteria are frequently almost identical in appearance, or else the slight differences are found not constant. In such cases cultures in fluids are frequently of great service. In a recent paper[4] before the American Association for the Advancement of Science, I called attention to two pathogenic forms identical microscopically and in their effects upon animals though found in widely separate sections of our country. One of the few differential characters was the presence of a membrane in the liquid cultures of one germ, its absence in the other.

The great importance of solid media in isolating bacteria and testing the purity of cultures is conceded by all engaged in this field of research. The advantage of starting cultures from a single germ by inoculating them from the colony, its progeny, is inestimable in obtaining accurate and reliable results. But that liquids have also a place, and a very important one, the facts above stated will, I hope, demonstrate without a doubt.

Botanical characters of the Black Rot, Physalospora Bidwellii Sacc.*

F. LAMSON SCRIBNER.

(WITH PLATE IX.)

The external characters of black rot of the grape are determined by the growth of the mycelium of *Phoma uvicola* B. & C., or, if we assume the mature form to be correctly determined, *Physalo-*

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[4] Medical News, 1886, Oct. 18.

* Read before Botanical Club, A. A. A. S., Buffalo meeting, 1886.

spora Bidwellii Sacc. As soon as the berry exhibits any signs of the malady, a thin section through the discolored part will show, under the microscope, the mycelium or vegetative portion of the fungus. This will be found near the surface of the berry and will be seen to consist of hyaline, colorless threads or hyphæ, of very irregular diameter (1–4μ), much branched and provided with more or less frequent septa. The very young branches remind one by their shape of the haustoria of *Peronospora*, but their position in respect to the cells of the host and their subsequent development reveal their true character. The presence of the septa is alone sufficient to distinguish the mycelium of the *Phoma* or *Physalospora* from that of *Peronospora*.

The mycelium traverses the tissues both between and through the cells and under its action the latter soon lose their turgescence and their contents turn brown; they gradually collapse and flatten, and the dried pulp remains only as a thin layer of tissue in which the vegetative part of the fungus occupies a large part.[1] During the earlier stages of the disease the mycelium is most abundant near the surface of the berry and here, at frequent points, just beneath the cuticle, it makes a condensed growth that results in the formation of the perithecia or conceptacles destined to contain the spores. At first colorless, the pseudo-parenchymatous tissue of these conceptacles soon become pale yellow, then brown and finally black. The conceptacles themselves are ovoid or globular bodies varying in size from 75μ to 140μ in diameter, and in their development they raise and finally burst through the cuticle, imparting to the surface of the berry a pimply or punctulous appearance. At the apex of the exposed part of each conceptacle there is a minute opening, or osteolum, through which the spores escape at maturity.

The microscope reveals the fact that the conceptacles are of two sorts—*pycnidia* and *spermagonia*—names determined by the character of their contents, otherwise they do not differ except in size, the pycnidia being the larger.

Pycnidia.—A cross-section of a pycnidium shows first a clear zone lining the cavity, consisting of very delicate tissue that gives rise to the short and thin walled threads, the *basidia*, upon which are borne the spores, in this case called *stylospores*, that completely fill the remaining portion of the cavity. These stylospores are one-celled, round or somewhat oblong, being in their longest diameter about 8μ. Under an amplification of 500 diameters the cell wall is clearly discernible, the contents having

[1] Viala and Ravaz, "Memoire sur une nouvelle maladie de la vigne, Le Black Rot," p 416.

a beaded appearance, or sometimes one or two nuclei in the otherwise clear contents are seen. These spores escape, probably by the absorption of water through the osteolum or opening at the apex of the pycnidium. Under certain conditions they issue as a minute worm-like thread, which is composed of vast numbers of spores glued or held together by a kind of mucilage. These threads which are more or less twisted are easily seen with a pocket lens. This manner of protusion is probably not constant, but exists under certain favoring conditions.

The stylospores germinate freely in water within a space of three or four hours. They throw out a slender tube which soon provides itself with septa, branches and quickly developes into a mycelium in every way like that seen within the tissues of the berry. How long these stylospores may retain their germinative power is unknown, but it is not likely that they hold it through the winter season.

SPERMAGONIA.—The spermagonia have exactly the appearance of the pycnidia but are usually smaller, they are also far less numerous. Within the outer wall there is a clear zone from which arise the very slender basidia that project radially toward the center of the cavity. The spores, or as they are termed, the spermatia, having been supposed to have a fertilizing power, are borne upon the summit of the basidia and when mature escape in vast number from the spermagonium through an opening at its apex. They are cylindrical, obtuse at each end, 5-8μ long and about 7μ in diameter. What may be the rôle of these spermatia in the economy of the fungus is a matter of speculation. In speaking of the nature of these bodies in general, in the order Pyrenomycetes, Cornu says they are true spores, since they germinate and give out filaments having all the appearance of mycelial threads. He regards them as very small conidia of a special form, borne upon particular arbuscles in protecting conceptacles. They do not in general germinate in pure water and they have a rather slow development; their physiological rôle appears to be determined by their very small size and the circumstances which their germination require.

It has been quite generally supposed that they constitute the male element in the process of reproduction, but there is no evidence to sustain this opinion. Their very small size and consequent lightness have suggested to the minds of some that their office is to more certainly effect the wide distribution of the fungus. It seems to have been conclusively shown that they are not the spores of a parasite on the Phoma.

The species of the genus Phoma are believed to represent

merely one stage or condition of certain ascigerous or ascosporous fungi, yet to be determined. Professor W. G. Farlow has very carefully described and illustrated the various conditions or spore-bearing forms of the fungus that causes the Black Rot of the plum and cherry trees, *Sphæria morbosa*. In this case there are shown pycnidia containing stylospores and spermagonia filled with spermatia, *conidia* produced externally on short stalks or *conidiophores*, and *sporidia*, which are spores formed in little sacs or asci within a perithecium. The last or ascigerous form is the mature or perfect state of the fungus. We have here four distinct varieties of supposed reproductive bodies, pycnidia, spermagonia, conidia and sporidia. In the black rot we have seen the first two upon the same mycelium and even associated in the same *stroma*, so that there is no possible doubt of their connection, and, reasoning from analogy, we would expect to find also the conidial and ascigerous forms.

CONIDIA.—I am confident that I have seen upon completely diseased berries gathered from the vine, but more particularly upon similarly diseased berries kept moist for a few days under a bell jar, the conidiophores of the Physalospora bearing imperfectly developed conidia. They certainly appeared to be growing from the exposed portion of the pycnidia, but whether from these or from specially formed sclerotia I am not prepared to say.

Messrs. Viala and Ravaz state that berries diseased with black rot, placed in the earth, have developed sclerotia; and, maintained in the soil at a temperature of 18° to 20° C., these sclerotia have produced conidioferous filaments. At Val Marie, upon the 17th of December, they observed sclerotia upon berries that had lain upon the ground for some time after being destroyed by this disease. These berries, placed in a moist atmosphere at a temperature of 20° to 22° C., produced the same conidioferous filaments.

The conidia serve to propagate the fungus, and consequently the rot which it occasions. If their development be delayed until spring, as perhaps it often is, a knowledge of their existence is particularly important, for by them the disease may be perpetuated from year to year.

SPORIDIA.—The discovery of the mature or ascigerous form of the so-called *Phoma uvicola*, or what it seems reasonable to assume to be such, is recorded by Mr. J. B. Ellis, of Newfield, New Jersey, in the Bulletin of the Torrey Botanical Club for August, 1880 (vol. vii, page 90). Mr. Ellis says that in the early part of May, 1880, Dr. E. C. Bidwell, of Vineland, New Jersey, informed him of having made this discovery on grapes which had

been diseased with the rot the season previous and were still hanging dry and shriveled on the vines. By way of experiment some of these berries were placed in water, where they were allowed to soak for three or four days. At the expiration of this time many of the perithecia (that before only contained Phoma spores) were now filled with well developed asci containing immature sporidia. Following up this discovery, Mr. Ellis at once searched for similar developments in shriveled grapes from his own vicinity. His efforts were rewarded by finding some ascigerous perithecia, together with an abundance of Phoma, on grapes gathered from the ground where they had probably lain through the winter.

Messrs. Viala and Ravaz did not succeed in finding, nor were they able to induce the development of the ascosporous form, neither have my efforts to this end met with better success. Thanks to the kindness of Mr. Ellis, who has very generously supplied me with specimens, I have been enabled to study its appearance and illustrate it.

The perithecia containing the asci are in all respects like those that enclose the stylospores, and they have every appearance of being developed from the same mycelium. The walls of the asci are very transparent, and it is difficult to determine their outline except they be separated and examined singly. They are cylindrical or subclavate, abruptly contracted at the base, obtuse at the summit, straight, or occasionally somewhat curved. Except for the sporidia they are perfectly transparent. Each ascus contains eight sporidia.

Mr. Ellis named this fungus Spheria Bidwellii, in honor of its discoverer; by a more recent classification it becomes *Physalospora Bidwellii* Sacc.[2] If we are right in our conclusions, we see that this parasite has four kinds of reproductive bodies: first, the stylospores, enclosed in conceptacles, together constituting the *Phoma uvicola* of authors; second, the spermatia produced at the same time and enclosed in similar though smaller conceptacles; third, the conidia externally developed on short conidiophores; and fourth, the sporidia which are formed in asci that are enclosed in a protecting perithecium. The stylospores (and possibly also the spermatia) are undoubtedly designed for the immediate propagation of the fungus. The conidia probably serve the same purpose, and by their tardy development may help to continue the fungus from year to year. The sporidia are without doubt the special reproductive bodies for the latter purpose. The mycelium

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[2] The description given by Ellis is as follows: "Peritheciis minutis globosis epidermidi tectis demum suberumpentibus, apice poro pertusis; ascis clavate-cylindraceis obtusis .0027 X.0005', sporidias octo, irregulariter ellipticas vel oblongas (continuas?), .0005-.0007' X.00015-.0002' foventibus; paraphysibus nullis."

within the diseased berries retains its vitality during the winter months and through the agencies of warmth and moisture of early spring and summer the asci and sporidia are produced.

The germination of these sporidia has never been observed, but if by any system of culture they can be made to reproduce the Phoma of the Black Rot their real nature will be settled beyond dispute.

EXPLANATION OF PLATE IX. Fig. 1. A fragment of epidermis of a diseased berry, showing five of the black "pustules" formed by the development of the pycnidia. From four of these slender, contorted, worm-like filaments are being extruded; these are the stylospores held together by a kind of mucilage.

Fig. 2. A section through a bit of the berry, including a pycnidium (P) and a spermagonium (S). At O is the osteolum of the pycnidium through which the spores escape at maturity.

Fig. 3. A section of a portion of a pycnidium, more highly magnified, showing the basidia.

Fig. 4. Three stylospores germinating.

Fig. 5. A section through the perithecium or conceptacle of the ascosporous form, showing the asci, etc.

Fig. 6. Two separate asci, showing the 8 sporidia in each.

Fig. 7. Four of the sporidia that have escaped from an ascus.

Fig. 8. An ascus, enclosing 8 sporidia, found June 2, 1886, in grape (destroyed in 1885 by "Black Rot") kept for a week in moist air. From camera lucida sketch made by Erwin F. Smith in the laboratory of the University of Michigan. Mr. Smith notes that the "receptacles containing the asci are numerous, and the asci themselves abundant."

Synopsis of North American Pines, based upon leaf-anatomy. II.*

JOHN M. COULTER AND J. N. ROSE.

8. **P. monophylla** Torr. & Frem. Section almost circular: stomata in 18–26 rows: number of ducts two[4] (.055–.115 mm.): number of cells in bundle-sheath 30–55: strengthening cells in fibro-vascular region: leaf 1 to 2 in. long.

In the Sierra Nevada and mountains of California.

The single leaf serves well to distinguish this species. It has been considered a single leaf or a connate pair, but its minute structure at once decides that it represents but one of the two leaves found in P. edulis, and the notion

*Continued from page 262.

[4] Dr. Engelmann, in *Bot. Calif.* ii. 124, says that the ducts vary from 2 to 14, but we have found but two. Our specimens have included the type.